

**Claims:**

1. An isolated, purified, or recombinant protein complex comprising:
  - (i) a tumor necrosis factor alpha (TNF- $\alpha$ ) polypeptide or a functional  
5 variant thereof;
  - (ii) a TNF- $\alpha$  receptor (TNFR) polypeptide or a functional variant thereof;  
and
  - (iii) at least one polypeptide selected from the group consisting of: NF- $\kappa$ B  
activating kinase (NAK), RasGAP3, TRCP1, TRCP2 and a  
10 functional variant thereof.
2. The complex of claim 1, wherein the TNFR polypeptide is a TNFR1 or TNFR2  
polypeptide.
- 15 3. The complex of claim 1, comprising a TNF- $\alpha$  polypeptide, a TNFR polypeptide  
and a NAK polypeptide.
4. The complex of claim 1, comprising a TNF- $\alpha$  polypeptide, a TNFR polypeptide  
and a RasGAP3 polypeptide.
- 20 5. The complex of claim 1, comprising a TNF- $\alpha$  polypeptide, a TNFR polypeptide  
and a TRCP1 polypeptide.
6. The complex of claim 1, comprising a TNF- $\alpha$  polypeptide, a TNFR polypeptide  
and a TRCP2 polypeptide.
- 25 7. The complex of claim 1, comprising a TNF- $\alpha$  polypeptide, a NAK polypeptide  
and a TNFR1 polypeptide.
- 30 8. The complex of any one of claims 1-7, further comprising at least one  
polypeptide selected from the group consisting of: TRADD, TRAF2, TRAP2  
and a functional variant thereof.

9. The complex of claim 8, comprising a TNF- $\alpha$  polypeptide, a NAK polypeptide, a TNFR1 polypeptide, a TRAF2 polypeptide and a TRADD polypeptide.
- 5 10. The complex of claim 8, comprising a TNF- $\alpha$  polypeptide, a TNFR polypeptide, a NAK polypeptide, a RasGAP3 polypeptide, a TRCP1 polypeptide, a TRCP2 polypeptide, a TRADD polypeptide, a TRAF2 polypeptide, and a TRAP2 polypeptide.
- 10 11. The complex of claim 1, wherein said TNF- $\alpha$  is a fusion protein.
12. The complex of claim 1, wherein said TNFR is a fusion protein.
13. The complex of claim 11 or 12, wherein said fusion protein comprises a domain  
15 that facilitates purification, isolation, or detection of said fusion protein.
14. The complex of claim 11 or 12, wherein said fusion protein comprises a domain selected from the group consisting of: affinity tags, radionucleotides, enzymes, and fluorophores.
- 20 15. The complex of claim 13, wherein said domain is selected from the group consisting of: polyhistidine, FLAG, Glu-Glu, glutathione S transferase (GST), thioredoxin, protein A, protein G, and an immunoglobulin heavy chain constant region.
- 25 16. The complex of claim 13, wherein said domain is FLAG.
17. An isolated, purified, or recombinant protein complex comprising:  
(i) a TNF- $\alpha$  receptor (TNFR) polypeptide or a functional variant thereof;  
30 and

(ii) at least one polypeptide selected from the group consisting of: NF- $\kappa$ B activating kinase (NAK), RasGAP3, TRCP1, TRCP2 and a functional variant thereof.

5 18. The complex of claim 17, comprising a TNFR polypeptide and a NAK polypeptide.

19. The complex of claim 17, comprising a TNFR polypeptide and a RasGAP3 polypeptide.

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20. The complex of claim 17, comprising a TNFR polypeptide and a TRCP1 polypeptide.

15 21. The complex of claim 17, comprising a TNFR polypeptide and a TRCP2 polypeptide.

22. The complex of claim 17, wherein said TNFR polypeptide is a TNFR1 polypeptide or a TNFR2 polypeptide.

20 23. The complex of any one of claims 17-22, further comprising at least one polypeptide selected from the group consisting of: TNF- $\alpha$ , TRADD, TRAF2, and TRAP2.

25 24. The complex of claim 23, comprising a TNF- $\alpha$  polypeptide, a TNFR polypeptide, a NAK polypeptide, a RasGAP3 polypeptide, a TRCP1 polypeptide, a TRCP2 polypeptide, a TRADD polypeptide, a TRAF2 polypeptide, and a TRAP2 polypeptide.

30 25. The complex of claim 17, wherein said TNFR polypeptide is a fusion protein.

26. The complex of claim 25, wherein said fusion protein comprises a domain that facilitates purification, isolation, or detection of said fusion protein.

27. The complex of claim 25, wherein said fusion protein comprises a domain selected from the group consisting of: affinity tags, radionucleotides, enzymes and fluorophores.
- 5 28. The complex of claim 26, wherein said domain is selected from the group consisting of: polyhistidine, FLAG, Glu-Glu, glutathione S transferase (GST), thioredoxin, protein A, protein G, and an immunoglobulin heavy chain constant region.
- 10 29. The protein complex of any one of claims 1 and 17, wherein at least one protein in said complex is labeled.
30. The protein complex of claim 29, wherein said label is a detectable label.
- 15 31. The protein complex of claim 29, wherein said label is selected from the group consisting of: polyhistidine, FLAG, Glu-Glu, glutathione S transferase (GST), thioredoxin, protein A, protein G, and an immunoglobulin heavy chain constant region.
- 20 32. An isolated protein complex comprising two or three polypeptides, the protein complex selected from the group consisting of:
- 25 (i) a complex of a fragment of TNF- $\alpha$  polypeptide, a TNFR polypeptide and a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2;
- (ii) a complex of a fragment of TNF- $\alpha$  polypeptide, a fragment of TNFR polypeptide and a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2;
- 30 (iii) a complex of TNF- $\alpha$  polypeptide, a TNFR polypeptide and a fragment of a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2;

- (iv) a complex of TNF- $\alpha$  polypeptide, a fragment of TNFR polypeptide and a fragment of a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2;
- 5 (v) a complex of a fragment of TNF- $\alpha$  polypeptide, a TNFR polypeptide and a fragment of a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2;
- (vi) a complex of a fragment of TNF- $\alpha$  polypeptide, a fragment of a TNFR polypeptide and a fragment of a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2;
- 10 (vii) a complex of a fragment of TNFR polypeptide and a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2;
- (viii) a complex of TNFR polypeptide and a fragment of a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and
- 15 TRCP2; and
- (ix) a complex of a fragment of TNFR polypeptide and a fragment of a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2.
- 20 33. A host cell comprising a first nucleic acid, a second nucleic acid and a third nucleic acid, wherein the first nucleic acid comprises a recombinant nucleic acid encoding a TNF- $\alpha$  polypeptide, wherein the second nucleic acid comprises a recombinant nucleic acid encoding a TNFR polypeptide and wherein the third nucleic acid comprises a recombinant nucleic acid encoding a polypeptide
- 25 selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2.
34. The host cell of claim 33, wherein the first nucleic acid comprises a recombinant nucleic acid encoding a TNF- $\alpha$  polypeptide, wherein the second nucleic acid comprises a recombinant nucleic acid encoding a TNFR1 polypeptide and wherein the third nucleic acid comprises a recombinant nucleic acid encoding a NAK polypeptide.
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35. A host cell comprising a first nucleic acid and a second nucleic acid, wherein the first nucleic acid comprises a recombinant nucleic acid encoding a TNFR, and wherein the second nucleic acid comprises a recombinant nucleic acid encoding a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2.
36. The host cell of claim 35, wherein the first nucleic acid comprises a recombinant nucleic acid encoding a TNFR1 polypeptide and wherein the second nucleic acid comprises a recombinant nucleic acid encoding a NAK polypeptide.
37. An assay for identifying a test compound which inhibits or potentiates the stability of a complex, comprising:
- (a) forming a reaction mixture including:
    - (i) a TNF- $\alpha$  polypeptide;
    - (ii) a TNFR polypeptide;
    - (iii) at least one polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2; and
    - (iv) a test compound; and
  - (b) detecting the presence of TNF- $\alpha$  or TNFR in the complex;
- wherein a change in the presence of TNF- $\alpha$  or TNFR in the complex in the presence of the test compound, relative to the presence of TNF- $\alpha$  or TNFR in the complex in the absence of the test compound, indicates that said test compound potentiates or inhibits the stability of said complex.
38. An assay for identifying a test compound which inhibits or potentiates the stability of a complex, comprising:
- (a) forming a reaction mixture including:
    - (i) a TNF- $\alpha$  polypeptide;
    - (ii) a TNFR polypeptide,
    - (iii) at least one polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2; and

- (iv) a test compound; and
- (b) detecting the association between the TNFR and a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2;
- 5 wherein a change in the association between TNFR and a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2 in the presence of the test compound, relative to the association between TNFR and a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2 in the absence of the test compound, indicates that said test
- 10 compound potentiates or inhibits the stability of said complex.

39. An assay for identifying a test compound which inhibits or potentiates the stability of a complex, comprising:
- (a) forming a reaction mixture including:
- 15 (i) a TNFR polypeptide;
- (ii) at least one polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2; and
- (iii) a test compound; and
- (b) detecting the association between the TNFR and a polypeptide selected
- 20 from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2;
- wherein a change in the association between TNFR and a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2 in the presence of the test compound, relative to the association between TNFR and a
- 25 polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2 in the absence of the test compound, indicates that said test compound potentiates or inhibits the stability of said complex.

40. A method for identifying a test compound which modulates activities of a
- 30 complex, comprising:
- (a) forming a protein complex comprising
- (i) a TNF- $\alpha$  polypeptide;

- (ii) a TNFR polypeptide,  
(iii) a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2; and
- (b) contacting the protein complex with a test compound, and
- 5 (c) determining the effect of the test compound for one or more activities selected from the group comprising (i) a change in the level of the protein complex, (ii) a change in the level of TNF- $\alpha$  or TNFR polypeptide in the protein complex, (iii) a change in the signaling enzymatic activity of the complex, or (iv) a change in the interaction between the TNF- $\alpha$  or TNFR polypeptide and
- 10 polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2.
41. A screening assay to identify compounds that inhibit or potentiate the stability of a complex, comprising
- 15 (i) providing a two-hybrid assay system including a first fusion protein comprising a TNFR polypeptide portion, and a second fusion protein comprising a portion of a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2, under conditions wherein said two hybrid assay is sensitive to interactions between the TNFR polypeptide and a polypeptide selected from the group consisting of:
- 20 NAK, RasGAP3, TRCP1, and TRCP2;
- (ii) measuring a level of interactions between said fusion proteins in the presence and in the absence of a test compound; and
- (iii) comparing the level of interaction of said fusion proteins,
- 25 wherein a decrease in the level of interaction is indicative of an compound that will inhibit the interaction between a TNFR polypeptide and a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2.
42. An isolated antibody, or fragment thereof, specifically immunoreactive with an
- 30 epitope of a polypeptide selected from the group consisting of: TNF- $\alpha$ , TNFR, NAK, RasGAP3, TRCP1, and TRCP2, wherein said antibody disrupts



formation of an interaction between TNF- $\alpha$  and a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2.

- 5 43. An isolated antibody, or fragment thereof, specifically immunoreactive with an epitope of a polypeptide selected from the group consisting of: TNF- $\alpha$ , TNFR, NAK, RasGAP3, TRCP1, and TRCP2, wherein said antibody disrupts formation of an interaction between TNFR and a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2.
- 10 44. A method for modulating, in a cell, a protein complex comprising at least a first protein and a second protein, wherein said first protein is TNFR, and wherein said second protein is selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2, said method comprising: administering to said cell a compound capable of modulating said protein complex.
- 15 45. The method of claim 44, wherein the protein complex further comprises TNF- $\alpha$ .
46. A method of producing a functional complex comprising:
- 20 (i) transfecting a cell with a polynucleotide encoding a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2;
- (ii) contacting said cell with a TNF- $\alpha$  polypeptide;
- (iii) thereby forming a complex.
- 25 47. The method of claim 46, further comprising a TNFR polypeptide.
48. A method for treating a TNF- $\alpha$ -related disorder, by administering an effective amount of a compound that inhibits the interaction of TNF- $\alpha$  or TNFR with a polypeptide selected from the group consisting of: NAK, RasGAP3, TRCP1, and TRCP2.
- 30 49. The method of claim 48, wherein said compound is selected from the group consisting of: a small molecule, an antibody, and a peptide.

50. A method of identifying a test compound that is a candidate modulator of inflammation or apoptosis, the method comprising:

5 (i) forming a mixture comprising a TRCP1 polypeptide or a variant polypeptide thereof, and a test compound; and

(ii) measuring the interaction between the TRCP1 polypeptide or the variant and the test compound;

wherein a test compound that interacts with the TRCP1 polypeptide or functional variant is a candidate modulator of inflammation or apoptosis.

10 51. The method of claim 50, wherein (i) comprises forming the mixture in vitro.

52. The method of claim 50, wherein (i) comprises contacting a cell expressing a TRCP1 polypeptide or a variant thereof, with the test compound.

53. A method of identifying a test compound that is a candidate modulator of inflammation or apoptosis, the method comprising:

15 (i) forming a mixture comprising a TRCP2 polypeptide or a variant polypeptide thereof, and a test compound; and

(ii) measuring the interaction between the TRCP2 polypeptide or the variant and the test compound;

20 wherein a test compound that interacts with the TRCP2 polypeptide or functional variant is a candidate modulator of inflammation or apoptosis.

54. The method of claim 53, wherein (i) comprises forming the mixture in vitro.

55. The method of claim 53, wherein (i) comprises contacting a cell expressing a TRCP2 polypeptide or a variant thereof, with the test compound.

25 56. A method of treating a TNF- $\alpha$ -related disease which includes an inflammatory or apoptotic component, by administering an effective amount of a therapeutic composition that modulates TRCP1.

57. A method of treating a TNF- $\alpha$ -related disease which includes an inflammatory or apoptotic component, by administering an effective amount of a therapeutic composition that modulates TRCP2.